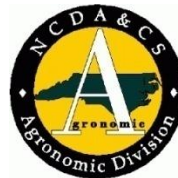


# Instruction for Pine-Wood Nematode Sampling and Assay

**Weimin Ye (Ph. D)**

Nematologist



**N.C. Department of Agriculture & Consumer  
Services, Agronomic Division, Raleigh, NC**

Physical Address: 4300 REEDY CREEK ROAD, RALEIGH NC 27607-6465

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Email: Weimin.Ye@ncagr.gov

**<http://www.ncagr.com/agronomi/>**

# Preface

The pinewood nematode (PWN) is the causal agent of pine wilt disease, one of the most damaging emerging pest problems to forests around the world. The international spread of PWN occurs mainly through the movement of infested logs, untreated wood products and wood-packaging material. PWN is native to North America where it causes relatively minor damage to native conifers. However, in many countries, it is a regulated pest and subject to quarantine because of its destructive potential. Exports of wood logs and commodities with softwood packaging materials now require a lab test for the presence/absence of this nematode species. The Agronomic Division of the N.C. Department of Agriculture & Consumer Services operates a high-throughput and publicly operated nematode assay lab. Recently, due to strict regulations on PWN, an increasingly large number of pinewood samples have been submitted to our lab. This document provides general guidelines about sample collection, submission and report retrieval.

# Southern yellow pine

1. Loblolly pine

2. Longleaf pine

3. Shortleaf pine

4. Slash pine



Major US exported pine species

# Eastern white pine

*Pinus strobus*



# Symptom caused by PWN



# Hosts of pine wood nematodes

- Red silver fir
- Balsam fir
- Grand fir
- Atlas cedar
- Deodar cedar
- European larch
- Tamarack
- Western larch
- Engelmann spruce
- White spruce
- Black spruce
- Red spruce
- Sitka spruce
- Armand's spruce
- Jack pine
- Arolla pine
- Sand pine
- Shore pine
- Lodgepole pine
- Japanese red pine
- Shortleaf pine
- Slash pine
- Limber pine
- Aleppo pine
- Jeffrey pine
- Korean pine
- Sugar pine
- Western white pine
- Mountain pine
- Bishop pine
- Austrian pine
- Longleaf pine
- Maritime pine
- Ponderosa/Yellow pine
- Colorado pine
- Monterey pine
- Red pine
- Northern pitch pine
- Pond pine
- Southwestern white pine
- Eastern white pine
- Scots pine
- Chinese pine
- Loblolly pine
- Japanese black pine
- Virginian pine
- Douglas fir
- Mountain hemlock

# PPQ Hypergeometric table

**TABLE 3-4-3: Hypergeometric Table for Random Sampling**

Total number of Inspectional units:	Randomly select this number of units to inspect:
1-13	Inspect all units
14-15	13
16-17	14
18-19	15
20-22	16
23-25	17
26-28	18
29-32	19
33-38	20
39-44	21
45-53	22
54-65	23
66-82	24
83-108	25
109-157	26
158-271	27
272-885	28
886-200,000	29

**95  
percent  
confidence  
level**

# PPQ Hypergeometric table

Sampling requirements for N.C., S.C., and V.A.\* **[LOGS ONLY]**

**95  
percent  
confidence  
level**

HYPERGEOMETRIC TABLE RANDOM SAMPLING	
Total number of Inspection units:	Randomly select this number of units to Inspect
1 - 24	Inspect all units
25	24
26	25
27	26
28	27
29	28
30	29
31	30
32-41	31
42-46	35
47-51	39
52-82	42
83-104	45
105-242	51
243-352	54
353-453	55
454-699	56
700-1,000	57
1001-4,999	58
5,000 and up	59
<b>95 % confidence of detecting a 5% infestation with 100% efficacy.</b>	
*	

## Harvest logs





## Nematode sampling



# Sampling

Bosch 36618-02 18-Volt 1/2-Inch  
Compact-Tough Lithium-Ion Drill/Driver with 2  
Slim Batteries



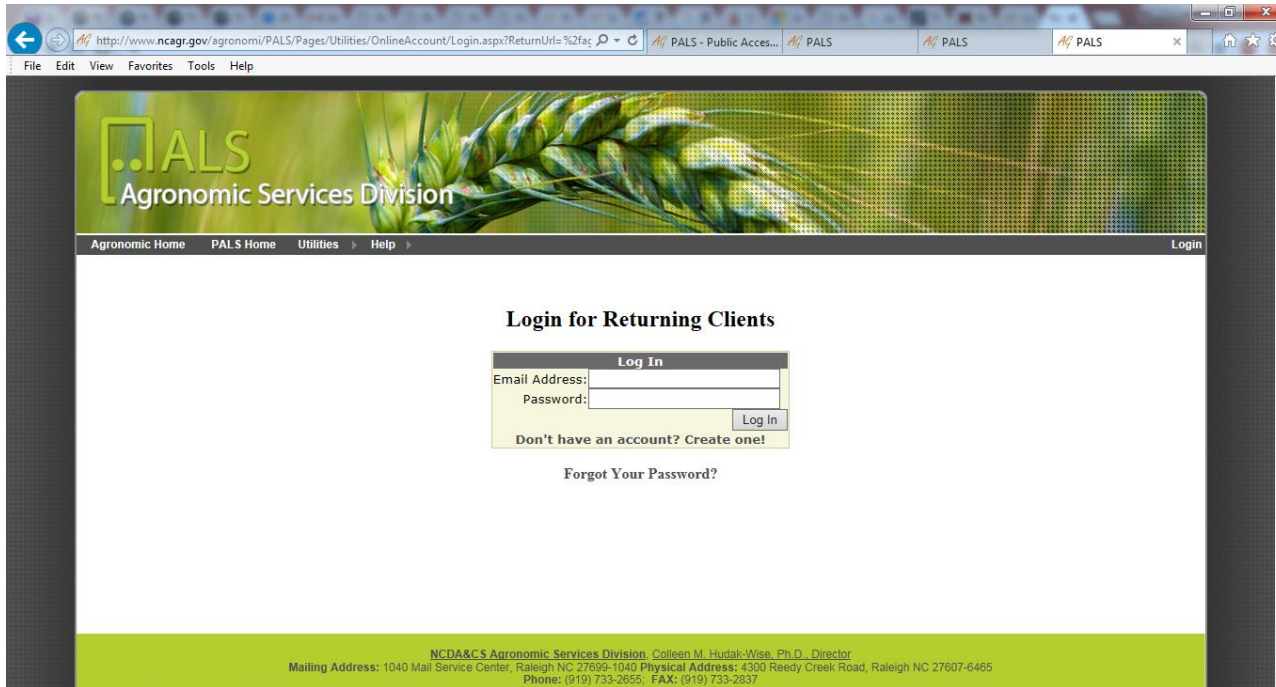
**IRWIN 2-1/8" Self Feed Bit**  
Item #: 154235 | Model #: 3046011



The PWN samples used were extracted from exported pine-wood logs. USDA/APHIS/PPQ officers followed the protocol provided in their Export Program Manual ([http://www.aphis.usda.gov/import\\_export/plants/manuals/domestic/downloads/xpm.pdf](http://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/xpm.pdf)) when inspecting and sampling PWN in pine-wood logs slated for export. The number of units to inspect was based on one of two hypergeometric tables at 95% confidence of detecting a 10% or 5% infestation with 100% efficacy, depending on the state. Two holes, up to six inches (15 cm) deep, were drilled per log at six inches (15 cm) from both ends using a 2.125-inch (5.4-cm), self-feeding-wood bit. The wood shavings from two logs were mixed together, and a minimum 200 g of wood shavings were collected as one lab sample. Samples were shipped overnight to NCDA&CS for nematode analysis.

# Prepare nematode submission on-line

<http://www.ncagr.gov/agronomi/PALS/Pages/Utilities/OnlineAccount/>



The screenshot shows a web browser window with the URL <http://www.ncagr.gov/agronomi/PALS/Pages/Utilities/OnlineAccount/Login.aspx?ReturnUrl=%2fag>. The page features a header with the PALS logo and "Agronomic Services Division". Below the header is a navigation bar with links: "Agronomic Home", "PALS Home", "Utilities", and "Help". The main content area is titled "Login for Returning Clients" and contains a "Log In" form. The form has fields for "Email Address:" and "Password:", a "Log In" button, and a link that says "Don't have an account? Create one!". Below the form is a link for "Forgot Your Password?". The footer contains contact information for the NCDA&CS Agronomic Services Division, including the name Colleen M. Hudak-Wise, Ph.D., Director, and the mailing address: 1040 Mail Service Center, Raleigh NC 27699-1040. It also lists the physical address: 4300 Reedy Creek Road, Raleigh NC 27607-6465, and phone/fax numbers: (919) 733-2655 and (919) 733-2837.

**Log In**

Email Address:

Password:

[Don't have an account? Create one!](#)

[Forgot Your Password?](#)

NCDA&CS Agronomic Services Division, Colleen M. Hudak-Wise, Ph.D., Director  
Mailing Address: 1040 Mail Service Center, Raleigh NC 27699-1040 Physical Address: 4300 Reedy Creek Road, Raleigh NC 27607-6465  
Phone: (919) 733-2655 FAX: (919) 733-2837

## On-line data entry instruction

<http://www.ncagr.gov/agronomi/documents/OnlineDataEntryNematologyPinewoodJune2018PALS.pdf>

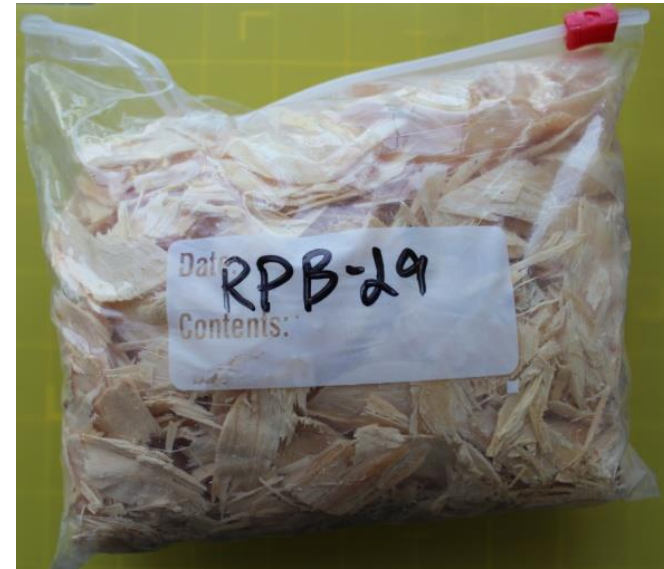
# Pine wood sample shipment

At least 200g per sample (no more than 300g)  
Put a wet paper towel inside the bag to keep the moisture  
Using one gallon ziploc bag

Pine wood nematode assay fee:  
\$10 per sample in-state (North Carolina)  
\$20 per sample out-of-state

Please submit sample information and  
pay fee on-line!

<http://www.ncagr.gov/agronomi/PALS/Pages/Utilities/OnlineAccount/Login.aspx?ReturnUrl=%2fagronomi%2fPALS%2fPages%2fUtilities%2fOnlineSubmission%2fLabAndType.aspx>



- Print on-line submission form, place in a plastic bag, and put in the shipment box with the samples. Do not put the sheet with the sample in the same bag.
- Ship the samples on the same day when samples are collected. Use express mail overnight service.
- Leave the samples indoor, keep moisture, avoid heat.
- Only live nematodes can be detected in the lab.

## Shipping address:

Dr. Weimin Ye  
Nematode Lab, Agronomic Division,  
North Carolina Department of Agriculture & Consumer Services  
Physical Address: 4300 Reedy Creek Road, Raleigh, NC 27607-6465

Phone: (919) 733-2655; FAX: (919) 733-2837

Email: [Weimin.Ye@ncagr.gov](mailto:Weimin.Ye@ncagr.gov)

<http://www.ncagr.gov/agronomi/nemhome.htm>



**Warning: Do not use Mailing Address: 1040 Mail Service Center, Raleigh, NC 27699-1040. This is in a different location. Any shipment received after 10am will be delivered to our lab the next day. We do not receive mail on the weekend and holiday.**

Go to PALS to check if your shipment is received the next day. It will take at least another day to receive the report. Email notification will be sent to you once report is completed.

# Nematode extraction



NCDA&CS Nematode assay lab: extraction of PWN  
Soak the wood sample in water in funnel overnight to get the nematodes

# Nematode extraction and identification



Sample is weighed



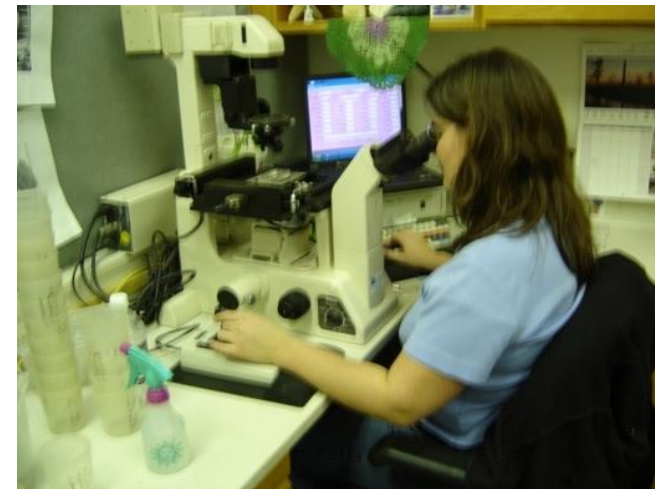
Soak in water



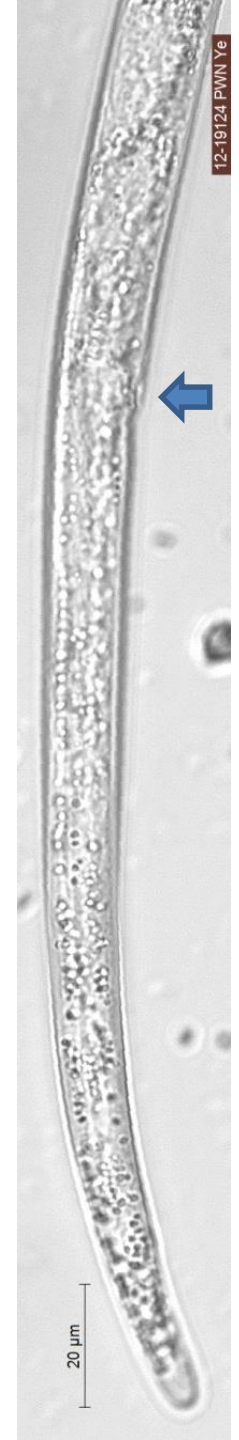
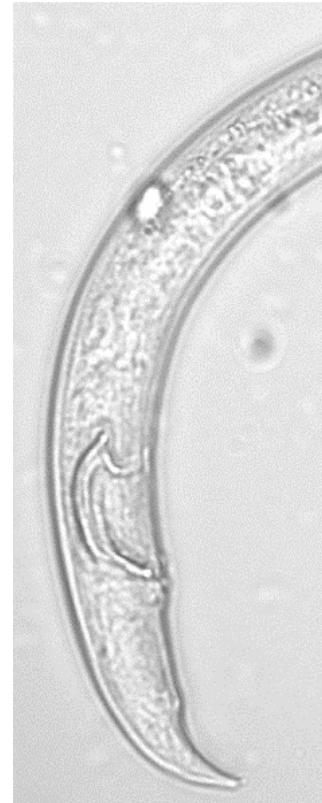
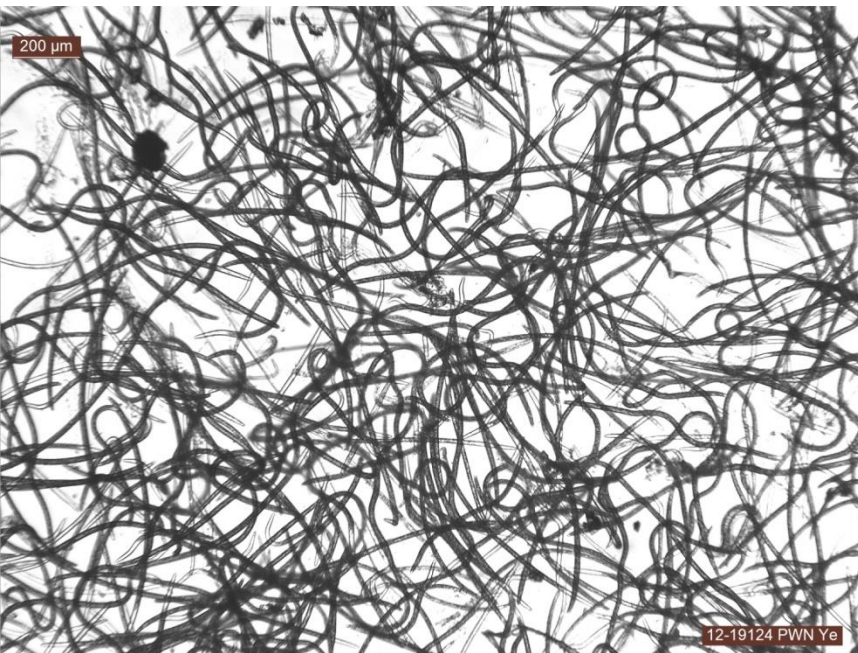
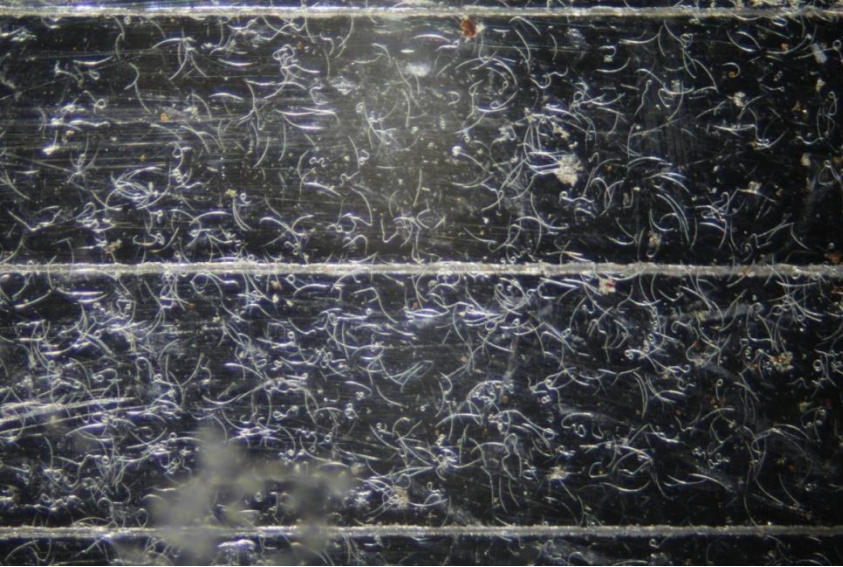
Collect nematode extraction  
in a cup the next day



Pour in a counting dish



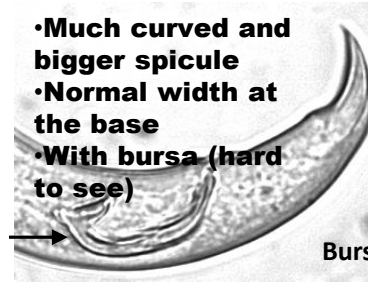
Identify nematodes in  
inverted microscope



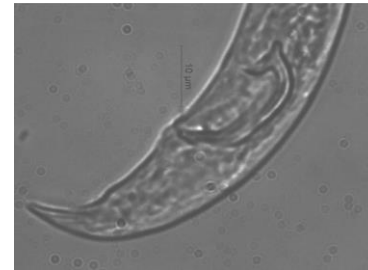
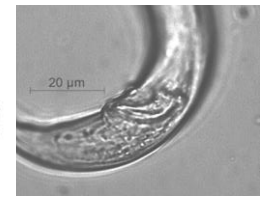
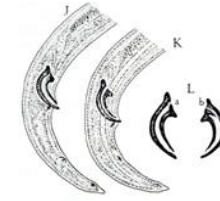
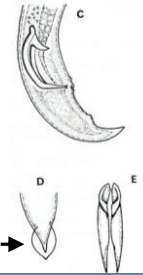
Micrographs of PWN from Japanese black pine (*Pinus thunbergii*) in Carteret County, N.C. (Lab ID: 12-19124)

# Pine wood nematode **Male** Aphelenchid (fungivore)

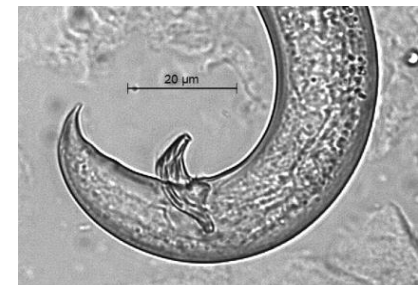
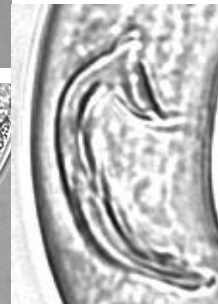
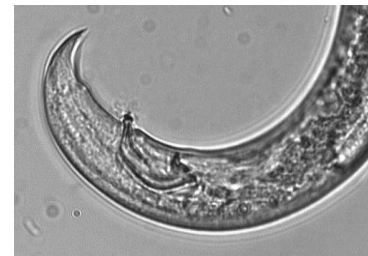
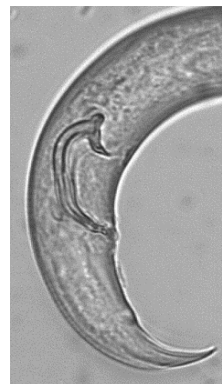
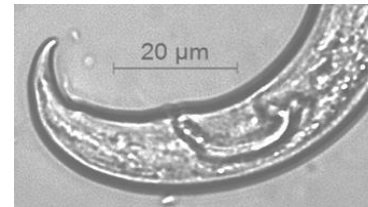
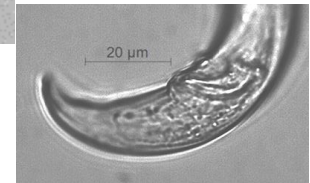
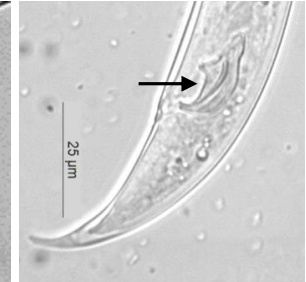
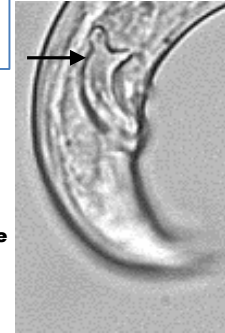
- Much curved and bigger spicule
- Normal width at the base
- With bursa (hard to see)



Bursa →



- Smaller, rose-thorn shaped spicule
- Much wider spicule at the base (see arrow)
- No bursa



Morphological comparisons on male between *Bursaphelenchus xylophilus* (left) and other wood-inhabiting aphelenchids (right)

# Sample submission

<http://www.ncagr.gov/agronomi/uyrnem.htm>

<http://www.ncagr.gov/agronomi/pdffiles/isnempd.pdf>

## Agronomic Services – Nematode Assay Forms & Information



Unless otherwise indicated, these forms are published in Adobe Acrobat (PDF) format.  
[Click here to download a free copy of Adobe Acrobat reader.](#)

### Agronomic Reports

[Find Your Nematode Assay Report \[not pdf\]](#)  
[Report Cover Sheets](#)  
[Understanding the Diagnostic Nematode Assay Report](#)  
[Understanding the Nematode Assay Report](#)

### Boxes

Sample boxes are not available by mail. Pick them up at your county [Cooperative Extension](#) office or at the [Agronomic Division](#) office in Raleigh.

### Brochures

[Nematode Assay](#)

### Instructional Presentation

[Nematode Management](#)

### Nema Notes:

[Note 1: Root-Knot Nematodes: Biocontrol with French Marigold](#)  
[Note 2: Nematode Management in Soybeans \(2-1 through 2-6\)](#)  
[Note 3: Root-Knot Nematodes on Tobacco \(3-1 through 3-10\)](#)  
[Note 3-11: Lesion Nematodes on Tobacco](#)  
[Note 4: Sampling Problems \(4-1 through 4-3\)](#)  
[Note 5: Control of Plant-Parasitic Nematodes on Strawberry](#)  
[Note 6: Nematode Problems in the Urban Landscape](#)  
[Note 7-1: Nematode Parasites of Corn](#)  
[Note 7-2: Nematode Parasites of Turf Grass](#)  
[Note 7-3: String Nematodes](#)  
[Note 8: The B Recommendation for This Field](#)  
[Note 9: Root-Knot Nematodes on Irish Potatoes](#)  
[Note 10: Nematode Problem Diagnosis \(10-1 through 10-2\)](#)  
[Note 11: Nematode Management in Cotton](#)  
[Note 12: Root-knot Nematodes on Vegetables](#)  
[Note 13: Apple Nematodes in Western North Carolina](#)  
[Note 14: Nematodes on Sweetpotato](#)  
[Note 15: Nematode Management on Peach](#)  
[Note 16: Nematode Management on Peanut](#)  
[Note 17: Managing Root-knot Nematode Species](#)  
[Note 18: Pinewood Nematode Sampling & Assay](#)  
[Note 19: Soybean Cyst Nematode \(\*Heterodera glycines\*\) Distribution in North Carolina, USA \*\*New!\*\*](#)

### Sample Information Forms Print, Fill Out by Hand & Submit via Mail

[Routine samples \(form AD-3\) \*\*Updated\*\*](#)  
[Continuation page for routine samples \(form AD-3+\)](#)  
[Diagnostic \(problem\) samples \(form AD-5\) \*\*Updated\*\*](#)  
[Continuation page for problem samples \(form AD-5+\)](#)  
[Phytosanitary certification \(form AD-14\)](#)

### Sampling Instructions & Protocols

[Sampling for Plant-Parasitic Nematodes](#)  
[Submitting Samples for Problem Diagnosis](#)  
[Pinewood Nematode Sampling and Assay Protocol](#)  
[Instructions for Online Data Entry for Pinewood Samples](#)  
[Instructions for Online Data Entry for Predictive Nematology](#)

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Use form AD-5 and AD 5+

On-line sample submission instruction

Form ADS (July 2018)

SAMPLE TYPE

Check one below

(Check one below)

# NEMATODE PROBLEM-DIAGNOSIS INFORMATION

NCDA&CS Agronomic Division Nematode Assay Section  
Mailing Address: 1040 Mail Service Center, Raleigh NC 27699-1040  
Physical Address (UPS/FedEx): 4300 Reedy Creek Road, Raleigh NC 27607  
Phone: (919) 733-2655 Web Address: [www.ncagr.gov/agronomi](http://www.ncagr.gov/agronomi)

FOR OFFICE USE ONLY

REPORT #

DATE REC'D

INITIAL

Submission ID. 5510



## SAMPLE INFORMATION

## PAYMENT

## GROWER INFORMATION (please print)

## CONSULTANT/OTHER RECIPIENT

<b>FARM ID</b> Use it for tracking this report	<b>FEE TOTAL</b> _____ <b>AMT PAID</b> _____	<b>LAST NAME</b> _____ <b>FIRST NAME</b> _____	<b>LAST NAME</b> _____ <b>FIRST NAME</b> _____
<b>SAMPLED BY</b> <input type="checkbox"/> Grower <input type="checkbox"/> NCDA&CS Agronomist <input checked="" type="checkbox"/> <b>Advisor</b> <input type="checkbox"/> Coop. Ext. Agent	<b>METHOD OF PAYMENT</b> ( ) CASH ( ) CHECK (payable to NCDA&CS) ( ) MONEY ORDER ( ) ESCROW (provide account name below) Put exporter's escrow account name	<b>BUSINESS NAME</b> _____ <b>ADDRESS</b> _____ Exporter's contact and email	<b>BUSINESS NAME</b> _____ <b>ADDRESS</b> _____ APHIS' contact and email
<b>SAMPLE DATE</b> _____		<b>CITY</b> _____ <b>STATE</b> _____ <b>ZIP</b> _____	<b>CITY</b> _____ <b>STATE</b> _____ <b>ZIP</b> _____
<b>COUNTY</b> _____ <b>STATE</b> _____ (where collected)		<b>PHONE</b> (____) _____ - _____	<b>PHONE</b> (____) _____ - _____
<b>NUMBER OF SAMPLES</b> _____		<b>E-MAIL</b> _____ <input type="checkbox"/> Do Not notify me when report is available.	<b>E-MAIL</b> _____ <input type="checkbox"/> Do Not notify me when report is available.

## FIELD HISTORY

No. (1 to 6)	LAB NUMBER (leave blank)	SAMPLE ID (5 characters only)	CURRENT CROP (Include variety, if known)	CROP LAST YEAR (Include variety, if known)	NEMATICIDE APPLIED LAST YEAR	SOIL TYPE	PLANT APPEARANCE (check any that apply)				SYMPTOM DISTRIBUTION	
							Normal	Stunted	Yellow	Dead	Entire	Patches
1		ID	Pine	Pine	Leave the other fields blank							
2		ID	Pine	Pine								
3		ID	Pine	Pine								
4		ID	Pine	Pine								
5		ID	Pine	Pine								
6		ID	Pine	Pine								

Thank you for using agronomic services to manage nutrients and safeguard environmental quality. — Steve Troxler, Commissioner of Agriculture

GROWER NAME (please print)

# **NEMATODE PROBLEM-DIAGNOSIS INFORMATION** *(continuation form)*



PAGE NUMBER

## FIELD HISTORY

#	LAB NUMBER (leave blank)	SAMPLE ID (6 characters only)	CURRENT CROP (include variety, if known)	CROP LAST YEAR (include variety, if known)	NEMATOCIDE APPLIED LAST YEAR	SOIL TYPE	PLANT APPEARANCE (check any that apply)				SYMPTOM DISTRIBUTION	
							Normal	Stunted	Yellow	Dead	Field	Patches
		ID	Pine	Pine								
		ID	Pine	Pine								
		ID	Pine	Pine								
		ID	Pine	Pine								

Thank you for using agronomic services to manage nutrients and safeguard environmental quality. — Steve Troxler, Commissioner of Agriculture

Access report on-line: <http://www.ncagr.gov/agronomi/PALS/Index.aspx>

Type your name as grower (exporter) or advisor (USDA/APHIS/PPQ)

Use the same name in information sheet, no password is required



NCDA&CS Agronomic Division

PALS Home Reports Samples Help Log in

### Report Search Result for Daewoo GTL

Customer: Daewoo GTL

12750 Center Court DR #460

Cerritos

☒ Client ☐ Advisor ☐ Agronomist

From: 2011 To: 2013

39 report(s) found.

[Download CSV](#)

[Download XML](#)

Fiscal Year	Report	Lab	Report Type	Status	Status Date	Farm ID	Number of Samples	PDF File	Spreadsheet	<input type="checkbox"/> Select All
2013	N001009	Nemato...	Pine Wood	Released	2012/10/29	ILM-T2-2012	4	<a href="#">New Report!</a>	<a href="#">Download Data</a>	<input type="checkbox"/>
2013	N001008	Nemato...	Pine Wood	Released	2012/10/29	ILM-T2-2012	25	<a href="#">New Report!</a>	<a href="#">Download Data</a>	<input type="checkbox"/>
2013	N000798	Nemato...	Pine Wood	Released	2012/10/11	SAA 016	4	<a href="#">View Report</a>	<a href="#">Download Data</a>	<input type="checkbox"/>
2013	N000797	Nemato...	Pine Wood	Released	2012/10/11	ILM S2 2012	14	<a href="#">View Report</a>	<a href="#">Download Data</a>	<input type="checkbox"/>



Pinewood

# Nematode Report

Client: Northern Forest Product  
800 Davis Dr,  
Lucedale, MS 39452  
Sampled County : OUT OF STATE  
Sampled State : Mississippi

Advisor: Steve Dillion  
USDA-APHIS-PPQ  
800 Davis Dr,  
Lucedale, MS 39452

Sampled: 07/13/2013

Received: 07/14/2013

Completed: 07/15/2013

Farm: Sun 02

[Links to Helpful Information](#)

Nematologist's Comments: Pine wood nematode (*Bursaphelenchus xylophilus*) was found from 5 out of 29 samples submitted.

Sample Information		Results				
Total # Samples on Report:		29				
Sample ID	Lab ID	Number of Pine Wood Nematodes (PWN)	Sample Weight (g)	PWN / gram Wood	Number of Fungivores	Number of Bacterivores
1	N000402	0	276	0	0	2
2	N000403	0	283	0	0	6
3	N000404	0	267	0	0	0
4	N000405	0	284	0	0	0
5	N000406	26	288	1	0	4
6	N000407	0	328	0	0	2
7	N000408	640	225	3	0	0
8	N000409	1336	268	5	0	0
9	N000410	54	294	1	2	8
10	N000411	0	215	0	0	0
11	N000412	0	242	0	0	2
12	N000413	0	282	0	0	0
13	N000414	12	250	1	0	0



Reprogramming of the laboratory-information-management system that makes this report possible is being funded through a grant from the North Carolina Tobacco Trust Fund Commission.

*Thank you for using agronomic services to manage nutrients and safeguard environmental quality.*  
- Steve Traxler, Commissioner of Agriculture.